SKEIKY and GUDERIAN Application No.: 09/684,215

Page 5

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,

Reg. No. 42,058

TOWNSEND and TOWNSEND and CREW LLP Two Embarcadero Center, 8<sup>th</sup> Floor

San Francisco, California 94111-3834 Tel: (415) 576-0200

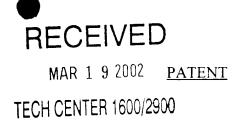
Fax: (415) 576-0300

ASP:dmw



SKEIKY and GUDERIAN Application No.: 09/684,215 Page 6



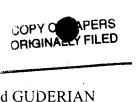


## **VERSION WITH MARKINGS TO SHOW CHANGES MADE**

## In the Specification:

Paragraph beginning at line 9 of page 2 has been amended as follows:

In one aspect, the present invention provides recombinant nucleic acid molecules that encode a fusion polypeptide, the recombinant nucleic acid molecules comprising a Ral2 polynucleotide sequence and a heterologous polynucleotide sequence, wherein the Ra12 polynucleotide sequence hybridizes to SEQ ID NO:3 under stringent conditions. In one embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence which is located 5' to a heterologous polynucleotide sequence. In another embodiment, the recombinant nucleic acid molecules further comprise a polynucleotide sequence that encodes a linker peptide between the Ra12 polynucleotide sequence and the heterologous polynucleotide sequence, wherein the linker peptide may comprise a cleavage site. In yet another embodiment, the recombinant nucleic acid molecules encode fusion polypeptides which further comprise an affinity tag. In yet another embodiment, the recombinant nucleic acid molecules encode a fusion polypeptide comprising a DPPD, a WT1, a mammaglobin, or a H9-32A heterologous polypeptide. In yet another embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence comprising at least about 30 nucleotides, at least about 60 nucleotides, or at least about 100 nucleotides. In yet another embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence as shown in SEQ ID NO:3. In yet another embodiment, the recombinant nucleic acid molecules comprise a Ra12 polynucleotide sequence that encodes a Ra12 polypeptide polynucleotide as shown in SEQ ID NO:4, SEQ ID NO:17 or SEQ ID NO:18.



SKEIKY and GUDERIAN Application No.: 09/684,215 Page 7



RECEIVED

MAR 1 9 2002 PATENT

TECH CENTER 1600/2900

Paragraph beginning at line 4 of page 4 has been amended as follows:

Figure 7 illustrates Ra12(short) polypeptide (SEQ ID NO:17), which has amino acids 1-30 of <u>SEQ ID NO:4-SEQ ID NO:3</u>.

Paragraph beginning at line 6 of page 4 has been amended as follows:

Figures 8 illustrates Ra12(long) polypeptide (SEQ ID NO:18), which has 128 amino acids amino acids 1-128 of SEQ ID NO:4.

Paragraph beginning at line 8 of page 4 has been amended as follows:

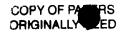
Figure 9 illustrates a construct of Ra12 (short) polynucleotide fused to a human mammaglobin gene (Met-His tag 6aa = SEQ ID NO:21).

Paragraph beginning at line 32 of page 15 has been amended as follows:

In one embodiment, the Ra12 polypeptide sequence is as shown in SEQ ID NO:4. In another embodiments, the Ra12 polypeptide sequence comprises a portion of SEQ ID NO:4. For instance, an Ra12 polypeptide comprising 30 amino acids (e.g., amino acids 1-30 of SEQ ID NO:4; SEQ ID NO:17) or an Ra12 polypeptide comprising 128 amino acids (e.g., 128 amino acids amino acids 1-128 of SEQ ID NO:4; SEQ ID NO:18) can be used as a fusion partner. See Examples 2 and 3 below.

Paragraph beginning at line 12 of page 21 has been amended as follows:

DPPD sequence was engineered for expression as a fusion protein with Ra12 by designing oligonucleotide primers to specifically amplify the mature secreted form. The 5' oligonucleotide containing an enterokinase recognition site (DDDK; SEQ)



SKEIKY and GUDERIAN Application No.: 09/684,215 Page 8





**PATENT** 

TECH CENTER 1600/2900

ID NO:22) has the sequences 5'-CAA TTA GAA TTC GAC GAC GAC GAC AAG GAT CCA CCT GAC CCG CAT CAG-3' (SEQ ID NO:15) and the 3' oligonucleotide sequence is 5'CAA TTA GAA TTC TCA GGG AGC GTT GGG CTG CTC (SEQ ID NO:16). The resulting PCR amplified product was digested with EcoRI and subcloned into the EcoRI site of the pET-Ra12 vector. Following transformation into the *E. coli* host strain (XL1-blue; Stratagene), clones containing the correct size insert were submitted for sequencing in order to identify those that were in frame with the Ra12 fusion. Subsequently, the DNA of interest (Fig. 3) was transformed into the BL-21 (pLysE) bacterial host and fusion protein expressed following induction of the culture with IPTG.

Paragraph beginning at line 5 of page 24 has been amended as follows:

In this example, a Ra12 polypeptide comprising 128 amino acids amino acids 1-128 of SEQ ID NO:4 was used as a fusion partner to link with the full length human mammaglobin gene. This long form of Ra12 polypeptide has the amino acid sequence shown in SEQ ID NO:18, and is referred to herein as "Ra12(long)". Cloning and expression procedures similar those described in Example 2 were used. Compared to a construct without a Ra12(long) sequence. the fusion construct with a Ra12(long) sequence substantially increased the expression of the fusion Ra12(long)-mammaglobin protein.

SF 1320995 v1